Organization TC1600 Bldg/Room REMSEN
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
If Undeliverable Return in Ten Days

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, $300

UNITED STATES POSTAGE U.S. OFFIC
PENALT
PRIVATE
PITNEY BO
02 1A $ 01
0004204479 AUG 23
MAILED FROM ZIP CODE

AN EQUAL OPPORTUNITY EMPLOYER

RECEIVED
SEP 05 2006
USPTO MAIL CENTER

BEST AVAILABLE COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

IFW

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/676,705	09/30/2003	Anna Marie Aguinaldo	A-71431-3	8128

7590 08/23/2006

Robin M. Silva, Esq.
Dorsey & Whitney LLP
Intellectual Property Department
Four Embarcadero Center, Suite 3400
San Francisco, CA 94111-4187

OIPE IAP4
SEP 05 2006
PATENT & TRADEMARK OFFICE

EXAMINER	
HISSONG, BRUCE D	
ART UNIT	PAPER NUMBER
1646	

DATE MAILED: 08/23/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/676,705	AGUINALDO ET AL.	
	Examiner	Art Unit	
	Bruce D. Hissong, Ph.D.	1646	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 June 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37* is/are pending in the application.

4a) Of the above claim(s) *6,8,9,13-26,29-34,36 and 37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5, 7, 10-12, 27-28, and 35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *sequence comparisons 1 and 2*.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group I, claims 1-29 and 35, and SEQ ID NO: 15, in the reply filed on 6/2/2006 is acknowledged.

2. Applicant's election with traverse of the specific modification/substitution F8E in the reply filed on 6/2/2006 is acknowledged. The traversal is on the ground(s) that the claimed mutations are presented in Markush format, and restriction among Markush group members is improper. The Applicants argue that the restriction requirement therefore be withdrawn, or alternatively, be held as an election of species.

These arguments have been fully considered and are not found persuasive. Each of the claimed mutations would result in a polypeptide with a different sequence, and different physical/biochemical characteristics. The MPEP, 806.04(b) states "Species may be either independent or related under the particular disclosure. Where species under a claimed genus are not connected in any of design, operation, or effect under the disclosure, the species are independent inventions." In the instant case, the claimed mutations produce polypeptides with a different sequence and therefore are not connected by design. Furthermore, it is noted that searching each of the claimed mutations, alone or in combination, represents an undue search burden because any search of a mutation(s) involves searching the mutation(s) itself, and the effect of the mutation(s) on the polypeptide.

The requirement is still deemed proper and is therefore made FINAL.

3. Claims 6, 8, 9, 13-26, 29-34, and 36-37 are therefore drawn to non-elected inventions, and are thus withdrawn. Therefore, claims 1-37 are currently pending, and claims 1-5, 7, 10-12, 27-28, and 35 are the subject of this office action.

Priority

The instant application, filed on 9/30/2003, claims benefit to provisional applications 60/489,725 (filed 7/24/2003), 60/477,246 (filed 6/10/2003), and 60/415,541 (filed 10/1/2002).

However, provisional application 60/415,541 does not specifically teach the F8E mutation in interferon (IFN)-β, or any other polypeptide, and thus does not provide support for the instant application. Accordingly, the earliest effective filing date of the instant application has been determined to be 6/10/2003.

Claim Objections

1. Claims 1-5 are objected to for reciting non-elected subject matter. Due to the election of SEQ ID NO: 15, the recitation of other SEQ ID NOs represents non-elected subject matter. Furthermore, claims 7 and 35 are objected to for depending from claim 1.

2. Claims 10-12 and 27-28 are objected to for reciting non-elected subject matter. Due to the election of the F8E modification, the recitation of other modifications represents non-elected subject matter.

3. Claim 12 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. In the instant case, due to the election of the F8E modification, both claim 11 and 12 read on a type I IFN variant comprising the F8E mutation.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1-5, 7, 10-12, 27-28, and 35 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. The claims are drawn to variant IFN proteins that may already be present in nature, and as written, do not show the "hand of man" in the inventive process. This rejection may be obviated by amending the claims to recite an "*isolated* variant".

Claim Rejections - 35 USC § 112, first paragraph - enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-5, 7, 10-12, 27-28, and 35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a variant IFN-β polypeptide comprising the F8E modification, does not reasonably provide enablement for all other possible IFN variants. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered when determining if the disclosure satisfies the enablement requirement have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breath of claims. Ex Parte Forman, (230 USPQ 546 (Bd. Pat. App. & Int. 1986); In re Wands, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988).

Claims 1-5, 7, and 10 are rejected due to the excessive breadth of the claims, which read on any possible variant of a type I IFN protein that exhibits increased solubility relative to a wild-type IFN protein. The breadth of the claims is also broad because the claims read on type I IFN variants that exhibit increased solubility in any type of medium/solution (e.g. water, physiological saline, lipids, etc). The claims are further drawn to a variant that retains at least one biological activity, exhibits reduced immunogenecity, and is incapable of dimer formation. Although the examples of the specification provide guidance for creating variant IFN polypeptides, there are no specific examples of any IFN-β polypeptide, or any other IFN polypeptide, that meets these claim limitations. Furthermore, claims 1-5 and 7 are excessively broad because they are directed to polypeptides that are "variants" of a type I IFN polypeptide, or differ from a naturally occurring IFN polypeptide by at least on substitution of a solvent-exposed residue (claim 3), or are "derived" from IFN-β, and as such, could read on substitution of all or most of the amino acid residues of a type I IFN polypeptide so long as at least one solvent-exposed residue is substituted. The specification does not provide guidance or

examples of any IFN polypeptide "variant" that is "derived" from IFN-β, other than the IFN-β polypeptides of the examples, and as stated above, does not show any polypeptide that retains at least one biological activity, exhibits reduced immunogenicity, and is incapable of dimer formation. A person of ordinary skill in the art would not be able to predict which amino acids, whether solvent-exposed/hydrophobic or otherwise could be substituted with any other amino acid and produce a variant polypeptide, or any polypeptide "derived" from IFN-β, with increased solubility. It is known in the art that even single amino acid changes or differences in the amino acid sequence of a protein can have dramatic effects on the protein's function. As an example of the unpredictable effects of mutations on protein function, Mickel *et al* (Med. Clin. North Am., 2000, Vol. 84(3), p. 597-607) teaches that cystic fibrosis is an autosomal recessive disorder caused by abnormal function of a chloride channel, referred to as the cystic fibrosis transmembrane conductance regulator (CFTR – p. 597). Several mutations can cause cystic fibrosis, including the G551D mutation. In this mutation, a glycine replaces the aspartic acid at position 551, giving rise to the cystic fibrosis phenotype. In the most common cystic fibrosis mutation, Δ-F508, a single phenylalanine is deleted at position 508, giving rise to the cystic fibrosis phenotype. Thus, even the substitution or deletion of a single amino acid can have dramatic and *unpredictable* effects on the function of the protein.

Therefore, without adequate guidance from the specification, one of ordinary skill in the art would require further, undue experimentation to produce an IFN polypeptide variant that is commensurate in scope with the claims of the instant invention. This is the case for variant IFN polypeptides comprising modifications at any base, as in claim 1, and also for IFN-β variants comprised of the specific modification sites recited in claim 10. Although claim 10 recites specific modification sites, a skilled artisan would still require further, undue experimentation to determine the effects of replacing the amino acids at the claimed positions with any of the recited amino acids. Finally, claims 11-12, 27-28, and 35 are rejected for depending from rejected base claims.

In summary, due to the excessive breadth of the claims, which read variant IFN polypeptides comprising a modification(s) at all possible amino acid positions, the lack of guidance and examples in the specification showing any IFN polypeptide variant, or any IFN polypeptide "derived" from IFN-β, or with unlimited modifications, that would produce a polypeptide that meets the limitations of the claims, and the unpredictability inherent in the art regarding the effects of modifying all possible amino acids residues of an IFN polypeptide, a

person of ordinary skill in the art would require further, undue experimentation to create a variant IFN polypeptide, other than a polypeptide comprising a F8E mutation of SEQ ID NO: 15, that is commensurate in scope with the claims of the instant invention.

Claim Rejections - 35 USC § 112, first paragraph – written description

Claims 1-5, 7, 10-12, 27-28, and 35 are rejected under 35 U.S.C. 112, first paragraph, containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-5, 7, and 10 are drawn to variant IFN polypeptides comprising *at least* one modification, wherein the variant polypeptide exhibits improved solubility relative to a wild-type IFN, retains at least one biological activity, differs from a naturally occurring IFN by at least one substitution of a solvent-exposed, hydrophobic residue, is incapable of dimer formation, and has reduced immunogenicity compared to a wild-type IFN. The claims do not require the variant IFN proteins of the instant invention to have any biological activity other that to retain at least one biological activity selected from immunomodulatory, antiviral, or antineoplastic activities, nor any particular structure other than comprising *at least* one modification/substitution at a solvent-exposed, hydrophobic residue. As stated in the preceding enablement rejection, the IFN variants or derivatives can be an IFN polypeptide substituted at any or all amino acid residues. The specification does not provide guidance or examples of any specific polypeptide, other than the IFN-β polypeptide of SEQ ID NO: 15 comprising a F8E substitution, that meets these claim limitations, and therefore the specification has not adequately described the genus of polypeptides that meet these limitations.

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof. In this case, the only factor present in the claims is a requirement that the variant IFN protein must retain at least one biological activity and comprise *at least* one modification of a solvent-exposed hydrophobic residue, or be otherwise "derived" from the IFN-β polypeptide of SEQ ID NO: 15. There is no identification of

any particular portion of any IFN variant protein that must be conserved in order to maintain function. Accordingly, in the absence of sufficient distinguishing characteristics, the specification does not provide adequate written description of the claimed genus.

Claim Rejections - 35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The metes and bounds of the term "incapable" are not defined by the claim, as there are known methods for artificially inducing polypeptides to form dimers (e.g. proteins dimerized by binding of an antibody). The Examiner suggest amending the claim to read, **as an example and without adding new matter**, "wherein said variant interferon does not naturally form dimers", or "wherein said variant interferon does not form dimers *in vivo*."

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

1. Claims 1-5, 27, and 35 are rejected under 35 U.S.C. 102(a) as being anticipated by Whitty *et al* (US 2002/0155547A1). The claims of the instant invention are drawn to a variant IFN protein that exhibits improved solubility relative to a wild-type IFN protein, maintains at least one biological activity, is incapable of dimer formation, exhibits reduced immonogenicity, and differs from a naturally occurring IFN by at least one substitution of a solvent-exposed hydrophobic residue. Whitty *et al* teach variants of IFN proteins, including IFN-β, wherein at

least one solvent-exposed hydrophobic amino acid residue is replaced by another amino acid residue (see paragraph 0092). Specifically, Table 1 discloses an IFN-β polypeptide in which the phenylalanine at position 8 is replaced with an alanine. Although Whitty *et al* does not specifically disclose the IFN-β variants as having improved solubility, reduced immuogenicity, maintaining at least one biological activity, or being incapable of dimer formation, it would be expected, in the absence of evidence to the contrary, that the IFN-β polypeptide disclosed by Whitty *et al* in Table 1 would inherently possess these features due to the replacement of the solvent-exposed phenylalanine at position 8. Because the USPTO does not have the facilities for testing the properties of the disclosed IFN-β variant of Whitty *et al*, the burden is on the applicant to show a novel and unobvious difference between the claimed IFN variant and that of the prior art. See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray, 10 USPQ 2d 1922 1923 (PTO Bd. Pat. App. & Int.). Therefore, the IFN-β variant disclosed by Whitty *et al* meets the limitations of claims 1-5 and 27 of the instant application. Furthermore, Whitty *et al* teaches "therapeutic compositions" of IFN variants (paragraph 0044), with said compositions comprising the IFN polypeptides and other physiologically compatible ingredients. Because "physiologically compatible ingredients" would encompass pharmaceutical carriers, Whitty *et al* also meets the limitations of claim 35 of the instant application.

2. Claims 1-5, 7, 10-12, 27-28, and 35 are rejected under 35 U.S.C. 102(a) as being anticipated by Pedersen *et al* (US 6,531,122). The subject matter of the claims of the instant invention is discussed *supra*. The claims are further drawn to an IFN variant derived from the sequence of SEQ ID NO: 15, wherein said variant results from replacing the phenylalanine at position 8 with another amino acid, specifically glutamic acid. Pedersen *et al* teaches IFN-β variants produced for the purpose of conjugation to various polymers. Specifically, Pedersen teaches replacement of various amino acids, including the phenylalanine at position 8 (F8), with other amino acids such as lysine (column 14, line 54 – column 15, line 20) or glutamic acid (column 17, line 58 – column 18, line 38). Thus, Pedersen *et al* discloses an IFN-β variant with an F8E substitution. Pedersen *et al* also teaches that the "parent" IFN-β has the sequence of SEQ ID NO: 2, which is 100% identical to the polypeptide of SEQ ID NO: 15 of the instant application (see sequence comparison 1, and column 9, lines 50-56). In additional, Pedersen *et al* discloses IFN-β molecules with decreased immunogenicity, and retaining biological activity (column 13, lines 16-38). Furthermore, even if Pedersen *et al* did not specifically teach reduced

immunogenicity and maintenance of biological function, it would be expected, in the absence of evidence to the contrary, that the IFN-β variants comprising the F8E substitution would inherently exhibit improved solubility relative to a wild-type IFN-β, maintain at least one biological activity, be incapable of dimer formation, and exhibit reduced immunogenicity compared to a wild-type IFN-β polypeptide because the IFN-β F8E polypeptide taught by Pedersen *et al* is identical to the F8E IFN-β polypeptide of the instant invention. Because the USPTO does not have the facilities for testing the properties of the disclosed IFN-β variant of Pedersen *et al*, the burden is on the applicant to show a novel and unobvious difference between the claimed IFN variant and that of the prior art. See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray, 10 USPQ 2d 1922 1923 (PTO Bd. Pat. App. & Int.).

Therefore, by teaching IFN-β variants derived from a sequence that is 100% identical to that of SEQ ID NO: 15, wherein the variants are characterized by replacement of F8 amino acid with a glutamic acid residue, Pedersen *et al* teaches an IFN-β F8E variant, and therefore meets the limitations of claims 1-5, 7, 10-12, and 27-28 of the instant application. Furthermore, Pedersen et al also discloses a variety of pharmaceutical compositions for administering IFN-β polypeptides and conjugates (column 38, line 47 – column 44, line 20), thus meeting the limitations of claim 35 of the instant application.

3. Claims 1-5, 7, 10-12, 27-28, and 35 are rejected under 35 U.S.C. 102(e) as being anticipated by Gantier *et al* (US 2004/0132977A1). The claims of the instant invention are drawn to variant IFN polypeptides, and with the election of SEQ ID NO: 15 and the F8E modification, are specifically drawn to a variant IFN-β polypeptide comprising a F8E modification. Claim 35 is further drawn to a pharmaceutical composition comprising a variant IFN protein and a pharmaceutically acceptable carrier. Gantier *et al* discloses a polypeptide, SEQ ID NO: 1122, that is 100% identical to the polypeptide of SEQ ID NO: 15 with a glutamic acid residue substituted for the phenylalanine at position 8 (see sequence comparison 2). Thus, by teaching an IFN-β polypeptide with a F8E modification/substitution, Gantier *et al* meets the limitations of claims 7, 10-12, and 27-28 of the instant application. Gantier *et al* also teaches pharmaceutical compositions of various modified polypeptides (see paragraph 0017), and thus also meets the limitations of claim 35. Although Gantier *et al* does not specifically teach an IFN variant that exhibits improved solubility relative to a wild-type IFN, retains at least one biological

activity, is incapable of dimer formation, differs from a naturally occurring IFN by at least one substitution of a solvent-exposed hydrophobic residue, or has reduced immunogenicity compared to a wild-type IFN, the F8E IFN-β polypeptide disclosed by Gantier *et al* would be expected to inherently meet these claim limitations because it is identical to the F8E IFN-β polypeptide of the instant application, and thus anticipates claims 1-5 of the instant application.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-5, 7, 10-12, 27-28, and 35 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-15 of copending Application No. 10/820,467. Although the conflicting claims are not identical, they are not patentably distinct from each other because both applications claim variant IFN polypeptides comprising modification at various residues, including substitution of a glutamic acid residue at position 8 of wild-type IFN-β. The claims of both applications also recite variant IFN proteins that exhibit increased solubility relative to a wild-type protein, and also exhibit reduced immunogenicity. Although the claims of the '467 application do not specifically recite variant IFN polypeptides that retain at least one biological activity, differ from naturally occurring IFN by at least one substitution of a solvent-exposed hydrophobic residue, or are incapable of dimer formation, the claimed IFN variants of the '467 application would be expected to

inherently possess these qualities. Therefore, it would be obvious to one of ordinary skill in the art to practice the invention of the instant application by following the claims and teachings of the '467 application. Finally, although the claims of the '467 application do not recite a pharmaceutical composition comprised of a variant IFN protein, it would be obvious to one of ordinary skill in the art to place any variant IFN polypeptide in a composition with a pharmaceutically acceptable carrier, and therefore claim 35 of the instant application is also obvious in view of the claims of the '467 application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

No claim is allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Bruce D. Hissong, Ph.D., whose telephone number is (571) 272-3324. The examiner can normally be reached M-F from 8:30am - 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol, Ph.D., can be reached at (571) 272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

BDH
Art Unit 1646

ROBERT S. LANDSMAN, PH.D
PRIMARY EXAMINER

SEQUENCE COMPARISON 1

RESULT 3
US-09-648-569A-2
; Sequence 2, Application US/09648569A
; Patent No. 6531122
; GENERAL INFORMATION:
; APPLICANT: Pedersen, A.H., et al.
; APPLICANT: Maxygen ApS
; TITLE OF INVENTION: Interferon-Beta Variants and Conjugates
; FILE REFERENCE: 0202us810
; CURRENT APPLICATION NUMBER: US/09/648,569A
; CURRENT FILING DATE: 2000-08-25
; NUMBER OF SEQ ID NOS: 45
; SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 2
; LENGTH: 166
; TYPE: PRT
; ORGANISM: Homo sapiens
US-09-648-569A-2

Query Match 100.0%; Score 874; DB 2; Length 166;
Best Local Similarity 100.0%; Pred. No. 1.1e-84;
Matches 166; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIY 60

Qy         61 EMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSL 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 EMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSL 120

Qy        121 HLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN 166
              ||||||||||||||||||||||||||||||||||||||||||||||
Db        121 HLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN 166
```

SEQUENCE COMPARISON 2

RESULT 1
US-10-658-834A-1122
; Sequence 1122, Application US/10658834A
; Publication No. US20040132977A1
; GENERAL INFORMATION:
; APPLICANT: Gantier, Rene
; APPLICANT: Guyon, Thierry
; APPLICANT: Drittanti, Lila
; APPLICANT: Vega, Manuel
; TITLE OF INVENTION: Rational Evolution of Cytokines for Higher Stability, Encoding Nucleic
; TITLE OF INVENTION: Acid
; TITLE OF INVENTION: Molecules and Related Applications
; FILE REFERENCE: 38751-922
; CURRENT APPLICATION NUMBER: US/10/658,834A
; CURRENT FILING DATE: 2003-09-08
; PRIOR APPLICATION NUMBER: 60/457,135
; PRIOR FILING DATE: 2003-03-21
; PRIOR APPLICATION NUMBER: 60/409,898
; PRIOR FILING DATE: 2002-09-09
; NUMBER OF SEQ ID NOS: 1306
; SOFTWARE: FastSEQ for Windows Version 4.0
; SEQ ID NO 1122
; LENGTH: 166
; TYPE: PRT
; ORGANISM: Homo sapiens
US-10-658-834A-1122

Query Match 100.0%; Score 873; DB 4; Length 166;
Best Local Similarity 100.0%; Pred. No. 5.1e-75;
Matches 166; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 MSYNLLGELQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MSYNLLGELQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIY 60

Qy         61 EMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSL 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 EMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSL 120

Qy        121 HLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN 166
              ||||||||||||||||||||||||||||||||||||||||||||||
Db        121 HLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN 166
```

Notice of References Cited	Application/Control No. 10/676,705	Applicant(s)/Patent Under Reexamination AGUINALDO ET AL.	
	Examiner Bruce D. Hissong, Ph.D.	Art Unit 1646	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-2002/0155547	10-2002	Whitty et al.	435/69.51
*	B	US-6,531,122	03-2003	Pedersen et al.	424/85.6
*	C	US-2004/0132977	07-2004	Gantier et al.	530/351
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Mickle JE et al. Genotype-phenotype relationships in cystic fibrosis. 2000. Med. Clin. North Am. Vol. 84, No. 3, p. 597-607.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

 0025-7125/00 $15.00 + .00

BEST AVAILABLE COPY

GENOTYPE-PHENOTYPE RELATIONSHIPS IN CYSTIC FIBROSIS

John E. Mickle, PhD, and Garry R. Cutting, MD

BEST AVAILABLE COPY

For inherited disorders, the interaction of three factors determines disease severity: (1) the nature of the defect in the responsible gene, (2) the context in which the defective gene operates (i.e., genetic background), and (3) the environmental influences. The contribution of the first component can be assessed by study of the relationship between gene defects and disease severity. Cystic fibrosis (CF) is an autosomal recessive disorder caused by abnormal function of a chloride channel called the *CF transmembrane conductance regulator (CFTR)*. Identification of the gene encoding *CFTR* and the discovery of numerous mutations in this gene have provided substantial data for genotype-phenotype analysis. Insight into this relationship has also been advanced by the discovery that patients with other disorders that clinically overlap with CF have mutations in each *CFTR* gene. Animal studies have shown the importance of genetic background. Emerging from this mosaic is a theme common to inherited disorders: Certain aspects of the CF phenotype are primarily determined by type of *CFTR* mutation, whereas some features are heavily influenced by other factors.

1. CF *is a variable disorder.* CF is a genetic disease of epithelia that is conspicuous in the lungs; pancreas; sweat glands; and, in men, vas deferens.[12,46] The CF phenotype is highly variable among unrelated individuals and within families. Lung disease is the primary cause of death in CF, but pulmonary manifestations show a high degree of interfamilial and intrafamilial phenotypic variability. Likewise, pancreatic disease ranges from complete loss of exocrine and endocrine functions in some CF patients, to partial pancreatic function in others, to pancreatitis only in others. Sweat gland dysfunction results in increased concentrations of

From the Institute for Genetic Medicine, Department of Pediatrics (JEM, GRC), and Department of Medicine (GRC), Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Material may be protected by copyright law (Title 17, U.S. Code)

sodium and chloride in sweat. The level of sweat chloride varies considerably among patients: from near-normal range, 40 to 60 mM/L, to 120 mM/L, with the average level being about 100 mM/L.[12, 66] Although useful for diagnostic purposes, abnormal sweat chloride concentrations do not cause illness. Male infertility is probably the most consistent feature of CF. Nearly all men with CF are infertile because of abnormalities in mesonephric duct–derived structures, the commonest of which is bilateral absence of the vas deferens.

2. *Epithelial electrolyte transport is abnormal in CF.* The clinical manifestations of CF are believed to be caused by abnormal electrolyte transport across epithelia leading to altered mucus viscosity and recurrent episodes of obstruction, inflammation, and progressive destruction of affected organs. For example, CF lung disease is thought to develop from the combination of absorptive and secretory defects.[53, 66] Altered electrolyte composition of airway surface fluid also affects the activity of antimicrobial peptides.[60] Loss of this activity has been proposed to underlie the predisposition to infection with pathogenic organisms, such as *Pseudomonas aeruginosa*. The importance of this pathophysiologic mechanism is unclear because precise salt concentration of airway surface fluid is a matter of some debate.[39, 66] Either way, defective electrolyte transport as a result of loss of cyclic adenosine monophosphate (cAMP)–activated chloride channels and hyperactivity of sodium channels in epithelial cells is the underlying metabolic derangement in CF.[63, 66]
3. *CFTR is defective in CF.* In CF epithelia, the defect in electrolyte transport is attributed to dysfunction of the CF transmembrane conductance regulator (*CFTR*).[32, 33] *CFTR* is expressed in a tissue-specific manner consistent with CF pathology.[11] In airway and intestinal epithelia, *CFTR* is localized to the apical membrane, whereas in the sweat duct it is present in the apical and the basolateral membranes. *CFTR* is an important component in the coordination of electrolyte movement across membranes of epithelial cells. Human *CFTR* is a 1480 amino acid integral membrane protein of the adenosine triphosphate (ATP)–binding cassette family.[59] *CFTR* is composed of two repeated motifs, each with a transmembrane domain (TMD) and a cytoplasmic nucleotide-binding fold (NBF) separated by a hydrophilic regulatory domain (R) (Fig. 1). The protein is a chloride

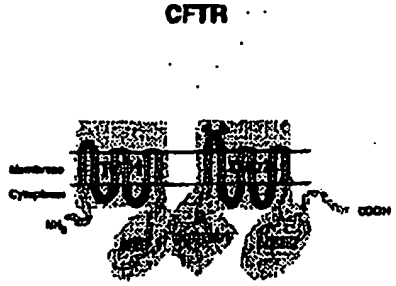

Figure 1. The cystic fibrosis transmembrane conductance regulator (*CFTR*). *CFTR* with the five domains indicated. TMD = Transmembrane domain; NBF = nucleotide binding fold; R = regulatory domain.

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

channel activated by cAMP-mediated PKA phosphorylation of the R domain and ATP binding and hydrolysis in the NBFs.[1,22,72] The activation and inhibition profiles of *CFTR* are typically used as reference points to evaluate the functional consequences of disease-associated mutations.

4. *CFTR regulates separate channels in the same cell.* *CFTR* is involved in ATP efflux and the concomitant regulation of outwardly rectified chloride channels (ORCCs).[22,23,32,36,39] Activation of ORCCs contributes to the whole-cell chloride conductance in epithelial cells. ORCCs have biophysical properties distinct from *CFTR*. Although protein that forms ORCCs has been reconstituted in planar lipid bilayers,[32] the genes encoding these channels have not been cloned. *CFTR* is also a regulator of the amiloride-sensitive epithelial sodium channel (ENaC).[21,62] In the absence of *CFTR*, ENaC is hyperactive, causing excessive absorption of sodium, increasing the difficulty of hydrating mucus secretions in the respiratory epithelia of CF patients. ENaC is composed of α, β, and γ subunits, for which the genes have been identified.[4,61]
5. *Mutations in CFTR cause CF.* More than 800 disease-causing mutations have been identified in the CFTR gene[39] (see also http://www.genet.sickkids.on.ca/cftr/). The mutation frequencies vary in relation to race and ethnicity. The common *CFTR* mutation ΔF508 is found on nearly 70% of CF chromosomes worldwide. An additional 20 mutations account for about 15% of CF alleles in white populations, whereas the remainder are rare mutations, occurring on only one or a few chromosomes.[46] To understand the consequences of *CFTR* mutations, two complementary approaches have been pursued. The first method involves analysis of specific *CFTR* mutations to determine the functional consequences for the development of genotype-based therapies. The second approach examines the relationship between genotype and phenotype to determine the clinical implications associated with mutations in *CFTR*. The particular insight derived from each approach is discussed here.

FUNCTIONAL CONSEQUENCES OF *CFTR* MUTATIONS

To provide a framework for understanding the consequences of mutations in *CFTR*, mutations are grouped into mechanistic classes based on demonstrated or predicted molecular dysfunction (Table 1).[46] Mutations affecting synthesis (class 1), maturation and trafficking (class 2), or activation (class 3) yield little or no functional protein and are usually associated with classic CF: elevated sweat chloride values, exocrine pancreatic deficiency, and obstructive pulmonary disease. Mutations that alter conductance (class 4) and abundance (class 5) diminish but do not eliminate *CFTR* function and are often associated with less severe phenotypes, such as pancreatic-sufficient CF or atypical CF with borderline sweat chloride levels. Mutations may also affect the ability of *CFTR* to regulate other channels (class 6), and loss of the regulatory activity may influence phenotype.[24]

An emerging concept of molecular pathophysiology is that mutations typically exert multiple effects. A mutation may cause more than one mechanistic error that contributes to loss of function and development of a phenotype. For example, the mutation G551D, which is associated with the classic CF phenotype, alters activation of CFTR (class 3) and affects the ability of *CFTR* to regulate separate channels (class 6).[1,24,46] Likewise, the common CF mutation ΔF508 alters

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

Table 1. CLASSIFICATION FOR *CFTR* MUTATIONS, MECHANISTIC DEFECTS, AND THERAPEUTIC APPROACHES

Class	Mutation	Defect	Therapy*
1	W1282X	Synthesis	Suppress stop mutations
2	ΔF508	Maturation	Chaperones, overexpression
3	G551D	Activation	Increase intracellular cAMP levels, regulate dephosphorylation
4	R117H	Conductance	Augment conduction
5	5T	Abundance	Increase mRNA and protein synthesis
6	ΔF508, G551D	Regulation	Block sodium channels, activate non-CFTR Cl^- channels

*Gene replacement therapy is also an option for each class.

processing (class 2) and the regulatory ability of *CFTR* (class 6). Therapeutic interventions have been developed to circumvent specific mechanistic defects. A major effort has been directed at overcoming the folding defect caused by the common mutation ΔF508. Two approaches have been chemical stabilization with glycerol and overexpression using sodium 4-phenylbutyrate.[70,71] Because optimal therapy may require restoration of *CFTR* chloride channel and regulatory functions, however, therapies aimed solely at overexpression of mutant *CFTR* in vivo may have different efficacy depending on the nature and location of the mutation. Other channels affected by *CFTR* dysfunction are also therapeutic targets. For example, *CFTR* is a regulator of ENaC. In the absence of *CFTR*, ENaC is hyperactive, causing excessive absorption of sodium. To prevent this excessive absorption, amiloride has been used to block the ENaC channels.[39] As such, combinational therapies may prove necessary to circumvent multiple mechanistic errors.

Two or more *CFTR* mutations that occur on the same chromosome *(in cis)* may act in concert to alter *CFTR* function and modify the CF phenotype. The complex allele R553Q-ΔF508 has been described to revert partially or ameliorate the phenotypic effects of ΔF508.[19,43] Likewise, other revertants (ΔF508-V1212I and R334W-R1158X) associated with mild or atypical CF have been described.[72,73] Two changes on the same allele, however, can elicit a more severe phenotype. For example, the mutation R117H occurs predominantly *in cis* with either the 5-thymidine (5T) or the 7-thymidine (7T) tract variant in intron 8 of *CFTR*.[28] These variants affect the efficiency of mRNA splicing for exon 9 in a tissue specific manner. The R117H-5T allele is associated with a pancreatic-sufficient (PS) CF phenotype. The R117H-7T allele is found in otherwise healthy men with congenital bilateral absence of vas deferens (CBAVD). Even though R117H contributes to *CFTR* dysfunction by altering conductance (class 4), the PS-CF and CBAVD phenotypes are differentiated by the poly-T variant, which affects *CFTR* abundance (class 5). Multiple mutations on the same allele effect different phenotypes, and analysis of *CFTR* mutations *in cis* provides a means to elucidate intramolecular interactions that affect phenotype.

A subcategory of mutations, polymorphisms, are relatively common in *CFTR*. By definition, a *polymorphism* occurs at a frequency of at least 1% in the general population, whereas rare mutations may be observed only once. Polymorphisms are common alterations, but their allelic frequencies often vary among populations. A single polymorphism is not considered sufficient to elicit a clinical phenotype as obligate heterozygotes, and unaffected individuals in the general population harbor single polymorphisms. *CFTR* bearing the polymor-

phism M470
pared with
occurs in NB
with disease
phenotype. N
with variable
polymorphis
different pop
olitis in Asia
Because allel
designed to
populations.

CLINICAL I

The rela
by two diffe
fied, and the
analysis has
disease. Alte
nity to exam
approach pr
For validatio
specific geno
Nearly
ΔF508 muta
form of the
pancreatic in
can be mild.
ing other ge
of other mu
CF patients
different CF
mutation A
pound hete
revealed bet
P. aeruginosa
suggest that
lands. Simil
A455E/ΔF5
and percent
drawn from
tion confers
alleles, such
associated w
Preserv
among affe
the *CFTR* lo
was strongl
this concept
the results
different mu

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

phism M470V displays altered processing and channel-gating properties compared with wild-type *CFTR* when expressed in heterologous cells.[11] M470V occurs in NBF1 along with more than 10 other polymorphisms. In combination with disease-associated mutations or other polymorphisms, M470V may affect phenotype. M470V in cis with the polymorphism F508C has been associated with variable penetrance of CBAVD.[43] The population-based variation of NBF1 polymorphisms may explain the increased incidence of related disorders in different populations when the genetic cause is unclear (*e.g.*, diffuse panbronchiolitis in Asian populations). Another consideration concerns pharmacogenetics. Because allelic variation affects differential drug interactions, pharmaceuticals designed to activate *CFTR* through NBF1 may be more efficacious in certain populations.

CLINICAL IMPLICATIONS OF *CFTR* MUTATIONS

The relationship between genotype and phenotype has been investigated by two different approaches. In the first approach, specific genotypes are identified, and the associated clinical manifestations are methodically delineated. Such analysis has prognostic implications for the pathophysiologic consequences of a disease. Alternatively, identification of a distinct phenotype provides an opportunity to examine the underlying genotypes. Genotypes uncovered by this second approach provide information relevant to the genetic cause of related disorders. For validation, both approaches require large numbers of patients with either a specific genotype or a distinct phenotype.

Nearly half of CF patients in the United States are homozygous for the ΔF508 mutation. In the homozygous state; ΔF508 is associated with the classic form of the disease, which includes significantly elevated sweat electrolytes, pancreatic insufficiency, and obstructive pulmonary disease that, in rare cases, can be mild.[12, 39] Clinical comparison of ΔF508 homozygotes with patients bearing other genotypes provides a means to determine the phenotypic consequences of other mutations. Because ΔF508 is relatively frequent, approximately 40% of CF patients are compound heterozygotes harboring ΔF508 on one allele and a different *CFTR* mutation on the other chromosome. In the Netherlands, the mutation A455E occurs at a relatively high frequency.[25, 26] Analysis of 33 compound heterozygotes carrying A455E on one allele and ΔF508 on the other revealed better pulmonary function tests and reduced rates of colonization with *P. aeruginosa* than ΔF508 homozygotes from the same population.[25] These results suggest that A455E produces less severe lung disease in patients in the Netherlands. Similarly a study of nine French-Canadian CF patients with the genotype A455E/ΔF508 revealed better pulmonary function (percent forced vital capacity and percent forced expiratory volume in 1 second) than five ΔF508 homozygotes drawn from the same population.[44] These studies indicate that the A455E mutation confers mild lung disease. A455E acts in a dominant fashion to the severe alleles, such as ΔF508. The same situation is observed for mutations that are associated with pancreatic sufficiency.

Preservation of pancreatic function was found to be highly concordant among affected siblings.[10] Because affected siblings have identical genotypes at the *CFTR* locus, this observation indicated that the nature of the CF mutation was strongly correlated with pancreatic status. Before cloning of the *CFTR* gene, this concept was supported by a study of DNA markers that flanked the gene; the results suggested that individuals who were pancreatic sufficient carried different mutations than those who were pancreatic insufficient.[10] Once the gene

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

was cloned, a subset of *CFTR* mutations was found to be associated with preserved pancreatic function.[40] A multicenter collaborative study confirmed this result but also emphasized that genotype is not completely predictive of pancreatic phenotype.[13] For example, most of 396 ΔF508 homozygotes were pancreatic insufficient, but 10 of these patients had preserved pancreatic function.[13] Similarly, patients carrying a mutation associated with preserved pancreatic function (R117H) were predominantly but not exclusively pancreatic sufficient.[13] Other mutations associated with mild pancreatic disease show a high but not exclusive association with preservation of pancreatic function. *CFTR*-bearing mutations associated with pancreatic sufficiency retain partial function, whereas nonfunctional mutations give rise to severe pancreatic disease.[39]

As with other features of the CF phenotype, sweat chloride concentrations can vary widely. The average sweat chloride concentration in CF patients is about 100 mM/L, but levels range from 60 mM/L to 160 mM/L.[33] Although most patients with CF have sweat chloride concentrations greater than 60 mM/L, a small fraction, approximately 1% to 2%, have a sweat chloride value in the normal range (i.e., <60 mM/L).[18] Although there is no clear correlation between the level of sweat chloride abnormality and severity of lung disease, there is evidence to suggest that patients with pancreatic sufficiency have less abnormal sweat chloride concentrations.[13,47] For instance, ΔF508/R117H compound heterozygotes have lower sweat chloride concentrations than age-matched and sex-matched ΔF508 homozygotes.[13] Because R117H is also associated with pancreatic sufficiency, this indicates that mutations producing mild pancreatic disease may be associated with less abnormal sweat chloride concentrations. This is not the case for all mutations, however. CF patients with the R334W mutation are frequently pancreatic sufficient but have sweat chloride concentrations similar to ΔF508 homozygotes.[22] The mutation G85E has been associated with mild and severe pancreatic disease. Some patients with G85E have low sweat chloride levels (<60 mM/L), whereas most appear to have levels comparable to ΔF508 homozygotes.[22,46] In CF patients with the G85E mutation, genetic background is likely a confounding factor that contributes to the clinical presentation.

One of the most consistent features of CF is male infertility, principally resulting from absence of the vas deferens bilaterally.[42,49] The fact that healthy male carriers of *CFTR* mutations (e.g., fathers of CF patients) are fertile indicates that male infertility occurs when *CFTR* activity falls below a 50% threshold. The *CFTR* genotypes 3849+10Kb C→T/ΔF508 and 3849+10Kb C→T/W1282X have been observed in a few fertile men with CF; here, fertility was attributed to 3849+10Kb C→T a mRNA splicing mutation because the ΔF508 and W1282X mutations were considered severe.[2,50] Some 3849+10Kb C→T compound heterozygotes were infertile, however. These observations suggest that the 3849+10Kb C→T mutation permits *CFTR* biosynthesis at or near the threshold level necessary to avoid infertility. Individual variation in proteins involved in the splicing process may affect the level of functional *CFTR* produced, accounting for the phenotypic discrepancy.

An alternate approach to genotype-phenotype studies involves characterization of distinct phenotypes and determination of the underlying genotypes. CBAVD is a distinct autosomal recessive disorder of infertility in otherwise healthy men that is estimated to affect 1 in 125.[8,49] Approximately 75% of men with CBAVD have *CFTR* mutations on both alleles.[3] Although not considered to be fully penetrant, a common variation in the gene that causes a mRNA splicing abnormality (5T) occurs on 12% of CBAVD alleles.[3] These data illustrate the phenotypic variability associated with mutations in the *CFTR* gene. Analysis of other CF-related disorders (*i.e.*, pancreatitis,[4,37] disseminated bronchiectasis,[23,24,51] and allergic bronchopulmonary aspergillosis[47]) has revealed similar results: A

diversity of see
mutations in CF
The high d
important in the
tion of phenoty
Genetic modifie
population stud
cosegregrating
amelioration of
ders.[40] This unl
the coordinatio
that the abnorm
part, by pharma
patients in Den
lectin (a lung su
infection) were
ensuing severity
provide a target
Assessing t
in human popu
often performed
homogeneous g
select *CFTR* mu
study of the co
As suspected fr
the severity of
problems with
to humans.[7,26] I
In mice, where
suggested that
cium-activated
ated chloride co
carrying a *CFT*
non-*CFTR* chlo
physiologic stat
the same as in
products in the
suggest that hu
mice. For exam
on human chro
reveals concord

SUMMARY

The genoty
monogenic dis
with the same
conclusions ca
alleles cause the
gosity for ΔF5
classic CF: obst
infertility, and

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

diversity of seemingly disparate clinical diseases are, in part, attributable to mutations in *CFTR*.

The high degree of variability in CF suggests that other factors must be important in the development of disease in the individual patient. The observation of phenotypic variability within affected sibships supports this contention. Genetic modifiers of the CF phenotype are evident from pedigree analysis, population studies, and animal models. The clinical investigation of a kindred cosegregrating CF and autosomal dominant polycystic kidney disease revealed amelioration of renal and hepatic manifestations for patients with both disorders.[62] This unique observation illustrates that *CFTR* is a critical component in the coordination of ion movement across epithelial membranes and suggests that the abnormal electrolyte transport characteristic of CF may be restored, in part, by pharmacologic regulation of separate ion channels. A study of 149 CF patients in Denmark revealed that certain allelic variants of mannose-binding lectin (a lung surfactant–like protein thought to provide protection from bacterial infection) were associated with an increased risk of bacterial infection and ensuing severity of lung disease.[27] Consequently, mannose-binding lectin may provide a target for therapeutic intervention.

Assessing the contribution of other genes to the CF phenotype is difficult in human populations because of a high degree of genetic diversity. Studies are often performed in animal models in which selective breeding can create more homogeneous genetic backgrounds to observe the phenotypic consequences of select *CFTR* mutations. The creation of numerous CF mouse lines facilitates study of the contribution of genetic background to CF phenotype in mice.[12, 17, 61] As suspected from human studies, the genetic background of mice can influence the severity of the disease, in the intestine and in the lungs.[6, 7, 34, 39] One of the problems with CF mice is that they do not develop a phenotype that is similar to humans.[7, 34] Intestinal obstruction leading to early death is a common feature in mice, whereas disease of the pancreas and lungs is minimal. It has been suggested that CF mice do not develop lung disease because endogenous calcium-activated chloride channels compensate for the deficiency in *CFTR*-mediated chloride conductance.[7] In support of this hypothesis, inbred congenic mice carrying a *CFTR* defect appear to develop lung disease as a result of lack of a non-*CFTR* chloride channel normally present in outbred mice.[34] Although the physiologic status of airway and intestinal epithelia in humans is not precisely the same as in mice, the murine studies illustrate the importance of other gene products in the development of lung and intestinal disease.[34] These studies suggest that human homologues likely exist for genetic modifiers identified in mice. For example, an intestinal modifier locus in mice is syntenic with a region on human chromosome 19, and segregation analysis with linked DNA markers reveals concordance among CF siblings.[72]

SUMMARY

The genotype-phenotype relationship in CF is complex despite its being a monogenic disorder. Factors that contribute to variability among individuals with the same genotype are an area of intense study. Nevertheless, certain conclusions can be derived from these studies. First, mutations in both CFTR alleles cause the CF phenotype. Homozygosity for ΔF508 or compound heterozygosity for ΔF508 and another severe mutation (*e.g.*, G551D, W1282X) cause classic CF: obstructive pulmonary disease, exocrine pancreatic deficiency, male infertility, and elevated sweat chloride concentrations. Clinical variability is →

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

observed among patients with the classic form of CF, especially with regards to the severity of lung disease. Although understanding of the role of other genes and environment in the development of lung disease is incomplete, evidence that other factors are important raises the possibility that therapeutic intervention may be possible at several levels. Second, genotype correlates more closely with certain features of the CF phenotype than others. Mutations that allow partial function of *CFTR* are often associated with pancreatic sufficiency, occasionally identified with normal sweat gland function, and sporadically correlated with mild lung disease. Partially functioning mutants rarely prevent maldevelopment of the male reproductive tract; an exception is 3849 + 10Kb C→T. These observations suggest that certain tissues require different levels of *CFTR* function to avoid the pathologic manifestations typical of CF. The genetic cause of several disorders that clinically overlap CF can be attributed, in part, to mutations in *CFTR*. Finally, molecular analysis of disease-associated mutations identified through genotype-phenotype studies provides a mechanistic framework for genotype-based therapeutic approaches and pharmaceutical interventions.

References

1. Anderson MP, Gregory RJ, Thompson S, et al: Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science 253:202–205, 1990
2. Augarten A, Kerem B, Yahav Y, et al: Mild cystic fibrosis and normal or borderline sweat test in patients with the 3849 + 10 kb C→T mutation. Lancet 342:25–26, 1993
3. Briel M, Gregor R, Kunzelmann K: Cl^- transport by cystic fibrosis transmembrane conductance regulator (CFTR) contributes to the inhibition of epithelial Na^+ channels (ENaC) in *Xenopus* oocytes coexpressing CFTR and EnaC. J Physiol 508:825–836, 1998
4. Canessa CM, Horisberger JD, Schild L, et al: Expression cloning of the epithelial sodium channel. Kidney Int 48:950–955, 1995
5. Chillón M, Casals T, Mercier B, et al: Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med 332:1475–1480, 1995
6. Clarke LL, Grubb BR, Gabriel SE, et al: Defective epithelial chloride transport in a gene-targeted mouse model of cystic fibrosis. Science 257:1125–1128, 1992
7. Clarke LB, Grubb BR, Yankaskas JR, et al: Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in CFTR (−/−) mice. Proc Natl Acad Sci U S A 91:479–483, 1994
8. Colin JA, Friedman KJ, Noone PG, et al: Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. N Engl J Med 339:653–658, 1998
9. Colin AA, Sawyer SM, Mickle J, et al: Pulmonary function and clinical observations in men with congenital bilateral absence of the vas deferens. Chest 110:440–445, 1996
10. Corey M, Durie P, Moore D, et al: Familial concordance of pancreatic function in cystic fibrosis. J Pediatr 115:274–277, 1989
11. Cuppens H, Lin W, Jaspers M, et al: Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes. J Clin Invest 101:487–496, 1998
12. Cutting GR: Cystic fibrosis. *In* Rimoin DL, Connor JM, Pyeritz RD (eds): Principals and Practices of Medical Genetics. New York, Churchill Livingstone, 1997, pp 2685–2717
13. Cystic Fibrosis Genotype/Phenotype Consortium: Correlation between genotype and phenotype in cystic fibrosis. N Engl J Med 329:1308–1313, 1993
14. DeBraekeleer M, Allard C, Leblanc J, et al: Genotype-phenotype correlation in cystic fibrosis patients compound heterozygous for the A455E mutation. Hum Genet 101:208–211, 1997
15. Delaney SJ, Alton E, Smith S, et al: Cystic fibrosis mice carrying the missense mutation G551D replicate human genotype-phenotype correlations. EMBO J 15:955–963, 1996
16. di Sant 'Agnese PA, Powell GP: The eccrine sweat defect in cystic fibrosis of the pancreas (mucoviscidosis). Ann N Y Acad Sci 93:555–599, 1962
17. Dorin JR,
18. Dork T,
19. Dork T,
20. Drumm
21. Duarte A,
22. Estivill X,
23. Fitzsimm
24. Fulmer S
25. Gan KH,
26. Gan KH,
27. Carred P,
28. Ciroldan
29. Cuggino
30. Highsmit
31. Ismailov
32. Jovov B,
33. Jovov B,
34. Kent G,
35. Kerem B
36. Kerem E,
37. Kerem E,
38. Kiesewet
39. Knowles
40. Kristidis

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

17. Dorin JR, Dickinson P, Alton EW, et al: Cystic fibrosis in the mouse by targeted insertional mutagenesis. Nature 359:211–215, 1992
18. Dork T, Dworniczak B, Aulehla-Scholz C, et al: Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Hum Genet 100:365–377, 1997
19. Dork T, Wulbrand U, Richter T, et al: Cystic fibrosis with three mutations in the cystic fibrosis transmembrane conductance regulator gene. Hum Genet 87:441–446, 1991
20. Drumm ML, Pope HA, Cliff WH, et al: Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. Cell 62:1227–1230, 1990
21. Duarte A, Amaral M, Barreto C: Complex cystic fibrosis allele R334W-R1158X results in reduced levels of correctly processed mRNA in a pancreatic sufficient patient. Hum Mutat 8:134–139, 1996
22. Estivill X, Ortigosa L, Perez-Frias J, et al: Clinical characteristics of 16 cystic fibrosis patients with the missense mutation R334W, a pancreatic insufficiency mutation with variable age of onset and interfamilial clinical differences. Hum Genet 95:331–336, 1995
23. Fitzsimmons SC: Cystic Fibrosis Foundation, Patient Registry 1996 Annual Report. Bethesda, MD, Cystic Fibrosis Foundation, 1997
24. Fulmer SB, Schwiebert EM, Morales MM, et al: Two cystic fibrosis transmembrane conductance regulator mutations have different effects on both pulmonary phenotype and regulation of outwardly rectified chloride currents. Proc Natl Acad Sci U S A 92:6832–6836, 1995
25. Gan KH, Heijerman HGM, Bakker W: Correlation between genotype and phenotype in patients with cystic fibrosis. N Engl J Med 330:865–866, 1994
26. Gan KH, Veeze HJ, van den Ouweland AMV, et al: A cystic fibrosis mutation associated with mild lung disease. N Engl J Med 333:95–99, 1995
27. Garred P, Pressler T, Madsen HO, et al: Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 104:431–437, 1999
28. Girodon E, Cazeneuve C, Lebargy F, et al: CFTR gene mutations in adults with disseminated bronchiectasis. Eur J Hum Genet 5:149–155, 1997
29. Guggino WB: Cystic fibrosis and the salt controversy. Cell 96:607–610, 1999
30. Highsmith WE Jr, Burch LH, Zhou Z, et al: A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. N Engl J Med 331:974–980, 1994
31. Ismailov II, Awayda MS, Jovov B, et al: Regulation of epithelial sodium channels by the cystic fibrosis transmembrane conductance regulator. J Biol Chem 271:4725–4732, 1996
32. Jovov B, Ismailov II, Benos DJ: Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. J Biol Chem 270:1521–1528, 1995
33. Jovov B, Ismailov II, Berdiev BK, et al: Interaction between cystic fibrosis transmembrane conductance regulator and outwardly rectified chloride channels. J Biol Chem 270:29194–29200, 1995
34. Kent C, Hes R, Bear C, et al: Lung disease in mice with cystic fibrosis. J Clin Invest 100:3060, 1997
35. Kerem BS, Buchanan JA, Durie P, et al: DNA marker haplotype association with pancreatic sufficiency in cystic fibrosis. Am J Hum Genet 44:827–834, 1989
36. Kerem E, Corey M, Kerem B, et al: The relation between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (ΔF508). N Engl J Med 323:1517–1522, 1990
37. Kerem E, Nissim-Rafinia M, Argaman Z, et al: A missense cystic fibrosis transmembrane conductance regulator mutation with variable phenotype. Pediatrics 100:E5, 1997
38. Kiesewetter S, Macek M Jr, Davis C, et al: A mutation in the cystic fibrosis transmembrane conductance regulator gene produces different phenotypes depending on chromosomal background. Nat Genet 5:274–278, 1993
39. Knowles MR, Church NL, Waltner WE, et al: A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. N Engl J Med 322:1087–1194, 1990
40. Kristidis P, Bozon D, Corey M, et al: Genetic determination of exocrine pancreatic function in cystic fibrosis. Am J Hum Genet 50:1178–1184, 1992

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

41. Linguegliа E, Volilley N, Waldman R, et al: Expression cloning of an epithelial amiloride-sensitive Na^+ channel. FEBS Lett 318:95–99, 1993
42. Macek M Jr, Mickle J, Vavrova V, et al: The identification of a possible revertant mutation (V1212) in two Czech ΔF508 homozygous siblings with cystic fibrosis and delayed onset of pancreatic insufficiency. Israel Journal of Medical Sciences 32:5182, 1996
43. McKusick VA: Mendelian Inheritance in Man. Baltimore, Johns Hopkins University Press, 1994
44. McNicholas CM, Nason MW Jr, Guggino WB, et al: A functional CFTR-NBF1 is required for ROMK2-CFTR interaction. Am J Physiol 97:F843–F848, 1997
45. Meschede D, Eigel A, Horst J, et al: Compound heterozygosity for the ΔF508 and F508C cystic fibrosis transmembrane conductance regulator (CFTR) mutations in a patient with congenital bilateral aplasia of the vas deferens. Am J Hum Genet 53:292–293, 1993
46. Mickle JE, Cutting GR: Clinical implications of cystic fibrosis transmembrane conductance regulator mutations. Clin Chest Med 19:443–458, 1998
47. Miller PW, Hamosh A, Macek M Jr, et al: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. Am J Hum Genet 59:45–51, 1996
48. O'Sullivan DA, Torres VE, Gabow PA, et al: Cystic fibrosis and the phenotypic expression of autosomal dominant polycystic kidney disease. Am J Kidney Dis 32:976–983, 1998
49. Phillipson G: Cystic fibrosis and reproduction. Reprod Fertil Dev 10:113–119, 1998
50. Pignatti PP, Bombieri C, Benetazzo M, et al: CFTR gene variant EVS8-5T in obstructive pulmonary disease. Am J Hum Genet 58:889–892, 1996
51. Pignatti PP, Bombieri C, Marigo C, et al: Increased incidence of cystic fibrosis gene mutations in adults with disseminated bronchiectasis. Hum Mol Genet 4:635–639, 1995
52. Rich DP, Anderson MP, Gregory RJ, et al: Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 347:358–363, 1990
53. Riordan JR, Rommens JM, Kerem BS, et al: Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. Science 245:1066–1073, 1989
54. Rozmahel R, Wilschanski M, Matin A, et al: Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. Nat Genet 12:280–287, 1996
55. Schwiebert EM, Egan ME, Hwang T, et al: CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. Cell 81:1–20, 1995
56. Schwiebert EM, Flotte TR, Cutting GR, et al: Both CFTR and outwardly rectifying chloride channels contribute to whole cell chloride currents. Am J Physiol 266:C1464–C1477, 1994
57. Sharer N, Schwarz M, Malone G, et al: Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. N Engl J Med 339:645–652, 1998
58. Sheppard DN, Rich DP, Ostedgaard LS, et al: Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. Nature 362:160–164, 1993
59. Sherif GE, Clarke LL, Boucher RC, et al: CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. Nature 363:263–266, 1993
60. Smith JJ, Travis SM, Greenberg P, et al: Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell 85:229–236, 1996
61. Snouwaert JN, Brigman KK, Latour AM, et al: An animal model for cystic fibrosis made by gene targeting. Science 257:1083–1088, 1992
62. Stutts MJ, Canessa CM, Olsen JC, et al: CFTR as a cAMP-dependent regulator of sodium channels. Science 269:847–850, 1995
63. Teem JL, Berger HA, Ostedgaard LS, et al: Identification of revertants for the cystic fibrosis delta F508 mutation using STE6-CFTR chimeras in yeast. Cell 73:335–346, 1993
64. Vazquez C, Antinolo G, Casals T, et al: Thirteen cystic fibrosis patients, 12 compound heterozygous and one homozygous for the missense mutation G85E: A pancreatic sufficiency/insufficiency mutation with variable clinical presentation. J Med Genet 33:820–822, 1996
65. Welsh MJ: ... 4:2718–2725,
66. Welsh MJ, ... al (eds): Th... McGraw-Hi...
67. Wilschanski ... tration with ... mutations. ...
68. Wine JJ: Th...
69. Wong PYD: ...
70. Zeitlin PL: ... 19:515–525,
71. Zeitlin PL: ... 452, 1999
72. Zielenski J. ... for meconiu...

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

65. Welsh MJ: Abnormal regulation of ion channels in cystic fibrosis epithelia. FASEB J 6:2718–2725, 1990
66. Welsh MJ, Tsui L, Boat TF, et al: Cystic fibrosis. *In* Scriver CR, Beaudet AL, Sly WS, et al (eds): The Metabolic and Molecular Basis of Inherited Disease, ed 7. New York, McGraw-Hill, 1995, pp 3799–3876
67. Wilschanski M, Zielenski J, Markiewicz D, et al: Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 127:705–710, 1995
68. Wine JJ: The genesis of cystic fibrosis lung disease. J Clin Invest 103:309–312, 1999
69. Wong PYD: CFTR gene and male infertility. Mol Hum Reprod 4:107–110, 1998
70. Zeitlin PL: Therapies directed at the basic defect in cystic fibrosis. Clin Chest Med 19:515–523, 1998
71. Zeitlin PL: Novel pharmacologic therapies for cystic fibrosis. J Clin Invest 103:447–452, 1999
72. Zielenski J, Corey M, Rozmahel R, et al: Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. Nat Genet 22:128–129, 1999

Address reprint requests to
Garry R. Cutting, MD
Institute for Genetic Medicine, CMSC 9-120
Johns Hopkins University School of Medicine
600 North Wolfe Street
Baltimore, MD 21287

BEST AVAILABLE COPY

Material may be protected by copyright law (Title 17, U.S. Code)

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☑ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☑ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.